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Mosaicism for the *FMR1* Gene Influences Adaptive Skills Development in Fragile X–Affected Males

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Fragile X syndrome is one of the most common forms of inherited mental retardation, and the first of a new class of genetic disorders associated with expanded trinucleotide repeats. Previously, we found that about 41% of affected males are mosaic for this mutation in that some of their blood cells have an active fragile X gene and others do not. It has been hypothesized that these mosaic cases should show higher levels of functioning than those who have only the inactive full mutation gene, but previous studies have provided negative or equivocal results. In the present study, the cross-sectional development of communication, self-care, socialization, and motor skills was studied in 46 males with fragile X syndrome under age 20 years as a function of two variables: age and the presence or absence of mosaicism. The rate of adaptive skills development was 2–4 times as great in mosaic cases as in full mutation cases. There was also a trend for cases with autism to be more prevalent in the full-mutation group. These results have implications for prognosis, for the utility of gene or protein replacement therapies for this disorder, and for understanding the association between mental retardation, developmental disorders, and fragile X syndrome. © 1996 Wiley-Liss, Inc.

KEY WORDS: fragile X syndrome, mosaicism, adaptive functioning, autism

INTRODUCTION

Fragile X syndrome is one of the most common forms of inherited mental retardation and affects an estimated 1/1,226 males. Carrier females are more prevalent, with estimates ranging from 1/259–1/700 [Brown and Jenkins, 1992]. The mutation responsible for the disorder was recently identified and was recognized as the first of a new class of genetic disorders with expanded trinucleotide repeats. In fragile X, the *FMR1* (fragile X mental retardation) gene contains a CGG repeat in its 5′ untranslated region. The CGG repeat number in the normal population varies from 6–54 copies. Male and female carriers of the "premutation" have about 55–200 copies of the repeat [Brown et al., 1993; Fu et al., 1991]. Affected males with the full mutation have >200 copies of the repeat and an associated hypermethylation in this region [Pieretti et al., 1991]. Because methylation prevents transcription of the gene, there is no *FMR1* protein in tissues of these full-mutation males. The function of the protein is unknown but may bind RNA [Ashley et al., 1993]. A case with the fragile X phenotype associated with a point mutation in the *FMR1* gene has also been described [De Boulle et al., 1993], which indicates that the lack of this one gene product is sufficient for the syndrome.

Among affected males, the degree of impairment in cognitive and adaptive functioning is broad, ranging from learning disability to profound retardation. Several studies have demonstrated an age-dependent reduction in IQ and adaptive behavior standard scores among affected males, suggesting a premature plateauing of cognitive [Lachiewicz et al., 1987] and adaptive skills [Dykens et al., 1993] development.

In addition to the variability in degree of impairment of this condition among males, there is also variability at the genetic level [Rousseau et al., 1993]. From 12 [Rousseau et al., 1993] –41% [Nolin et al., 1994] of affected males have two distinct cell populations in their tissues. These males are termed "mosaics," meaning that some of their cells have an unmethylated *FMR1* gene, whereas other cells have a nonfunctional, full-mutation gene. Consequently, mosaics, unlike full-mutation males, do produce some *FMR1* protein [Verheij et al., 1993]. This raises an important question: is there a correlation between the molecular

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status of an individual and his level of functioning? Specifically, do mosaic males, who produce some protein, exhibit higher levels of functioning than full-mutation males who produce no *FMR1* protein?

Three studies which examined whether or not IQ is affected by mosaicism have provided conflicting results. DeVries et al. [1993] found no relationship between presence or absence of premutation alleles in leukocyte cells and IQ (Stanford-Binet or Terman-Merrill tests) in 35 males, age 2–26 years. Similarly, Rousseau et al. [1993], in a collaborative study, observed *no differences* in degree of mental retardation between mosaic or full-mutation males. However, McConkie-Rosell et al. [1993] reported a correlation between methylation status and cognitive functioning in one unusual fragile X family with 6 adult brothers.

Complicating this scenario is the fact that fragile X males have been noted to demonstrate high levels of avoidance behavior (including gaze aversion) and arousal during social interactions [Cohen et al., 1988, 1989, 1991; Belser and Sudhalter, 1995], behaviors which are highly likely to be counterproductive during standardized IQ testing and which may therefore make interpretation of test data equivocal.

Consequently, in the present study, the rate of progress of adaptive skills (communication, daily living skills, socialization, and motor skills) development of fragile X males served as the dependent variable of interest, rather than IQ. We felt that adaptive skills provide a more realistic measure of functioning for these individuals, since they are obtained by interviewing caregivers who have the opportunity to observe their children in familiar surroundings.

SUBJECTS AND METHODS

Forty-six males with fragile X syndrome, ranging in age from 1 year, 11 months to 19 years at the time of their initial evaluation, were selected at random from our subject registry for participation in the present study. Cases over 20 years old were excluded in order to eliminate effects due to cessation of educational programs and/or institutionalization. Twenty-eight cases were found to have a mosaic DNA pattern. Mean (SD) ages and sample sizes were 8.82 (5.02) years, n = 28, for mosaic cases, and 9.39 (5.10) years, n = 18, for full-mutation cases. Mean age did not significantly differ between groups. All cases were residing in noninstitutional environments.

Adaptive Skills Assessment

Adaptive skills data were obtained on all individuals by interviews with caregivers (usually the mother) using the Vineland Adaptive Behavior Scales [Sparrow et al., 1984]. All interviews were performed by psychologists (usually I.L.C. or V.S.). The Vineland test provides standard scores and age equivalents in four domains: communication (receptive, expressive, and written); daily living skills (personal care, domestic skills, and community skills); socialization (interpersonal relationships, play and leisure, and coping skills) and motor skills (gross and fine motor skills). Only age-equivalents were examined, however, because of problems in interpreting standard scores on the Vineland scales [Silverstein, 1986]. Motor skills estimates were missing in 3 mosaic cases.

Statistical Analysis

The dependent variable of interest was the rate of change in age-equivalent scores with age across the two groups. The difference between regression slopes across groups was compared using F-tests of the interaction of mosaicism with age, as well as by comparison of the relative sizes of correlation coefficients across groups. Significance tests were one-tailed, since it was predicted that age-equivalents should increase with age at a greater rate in the mosaic cases.

DNA Analysis

Southern blot analysis was carried out according to procedures previously described [Nolin et al., 1994].

RESULTS

F-tests revealed significant slope differences across groups for communication ($F(1,42) = 6.94$, $P < .006$), daily living skills ($F(1,42) = 7.92$, $P < .004$), and socialization ($F(1,42) = 8.13$, $P < .004$). These effects are shown in Figures 1–3, which display the cross-sectional changes in adaptive skills with age for the two groups along with their corresponding regression equations and the 95% confidence limits for the slopes of the regression lines. Note that due to similarities in intercepts but differences in slope across groups, differences in level of functioning were not evident until about age 8–10 years.

On the Vineland scales, motor skills have a ceiling age-equivalent level of 6 years. Examination of the data relating motor skill age-equivalents to age revealed that few cases ever reached this ceiling (those who did were usually in the mosaic group). Further, there was no significant difference in variance in motor skills scores across groups ($P < .37$). Therefore, the same analysis was applied to this data set. Differences in rate of development of motor skills across groups did not reach significance ($F(1,39) = 1.70$, $P < .11$).

For the mosaic group, Pearson correlations between age and communication, daily living skills, socialization, and motor skills age-equivalents were as follows: $r = .75$ ($P < .0001$), $r = .81$ ($P < .0001$), $r = .72$ ($P < .0001$), and $r = .65$ ($P < .00025$), respectively. For the full-mutation group, Pearson correlations between age and communication, daily living skills, socialization, and motor skills age-equivalents were as follows: $r = .44$ ($P < .035$), $r = .62$ ($P < .003$), $r = .43$ ($P < .04$), and $r = .48$ ($P < .025$), respectively.

The regression equations shown in the figures indicate that slopes of functions for the mosaics were approximately 2–4 times as large as slopes for those who had the full mutation. All slopes were <1.0, indicating a subnormal rate of progress, and not a loss of adaptive skill development, with age.

Social skills emerged as an asset in the mosaic subtype, showing a faster rate of change with age relative to other areas of functioning. Specifically, the social

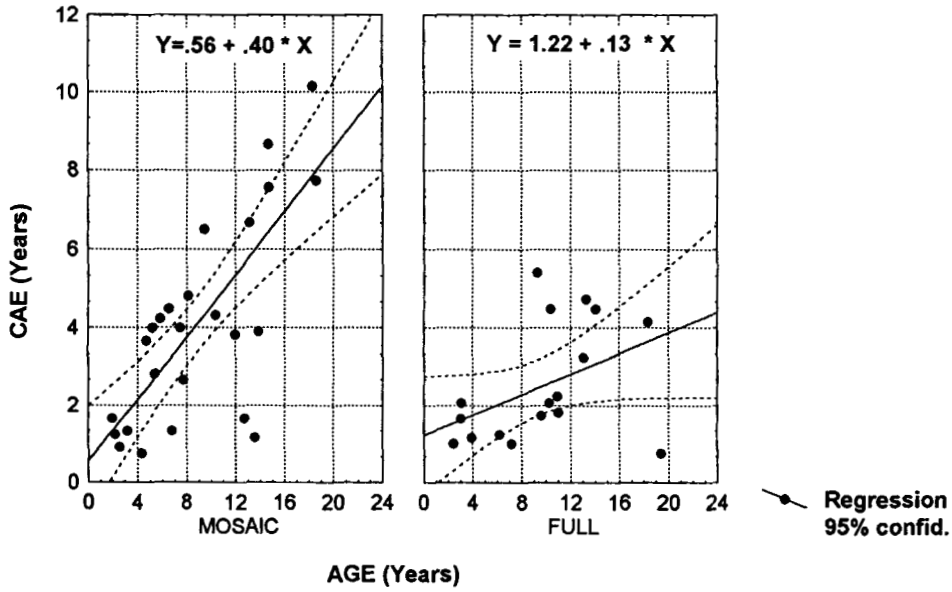

Fig. 1. Communication age-equivalents (CAE) as a function of age are shown for mosaic and full-mutation males. Solid lines and dashed lines show the best-fitting linear regression and 95% confidence limits for the slopes of the lines, respectively.

skills slope for the mosaic groups was four times that of the full-mutation group, with some older mosaic individuals demonstrating near age-appropriate socialization, as shown in Figure 3. By contrast, there was a trend for autism, a disorder characterized by marked impairment in social abilities, to be more frequently diagnosed among full-mutation (44%) than mosaic (18%) cases ($\chi^2(1) = 2.62$, $P < .11$, two-tailed test). These cases with autism had been diagnosed based on DSM-III-R [APA, 1987] criteria prior to knowledge of their mosaic status.

DISCUSSION

These results indicate that mosaicism (determined here in a categorical manner) has a strong influence over adaptive skills development when age-equivalents regressed against age are examined. Whether similar effects will hold for IQ is unclear. In the study by

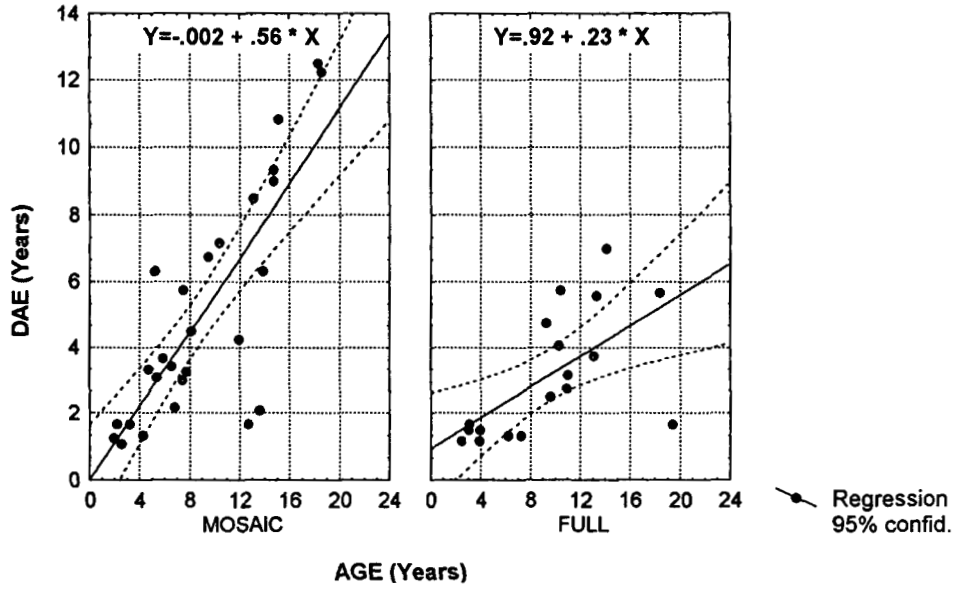

Fig. 2. Daily living skills age-equivalents (DAE) as a function of age are shown for mosaic and full-mutation males. Solid lines and dashed lines show the best-fitting linear regression and 95% confidence limits for the slopes of the lines, respectively.

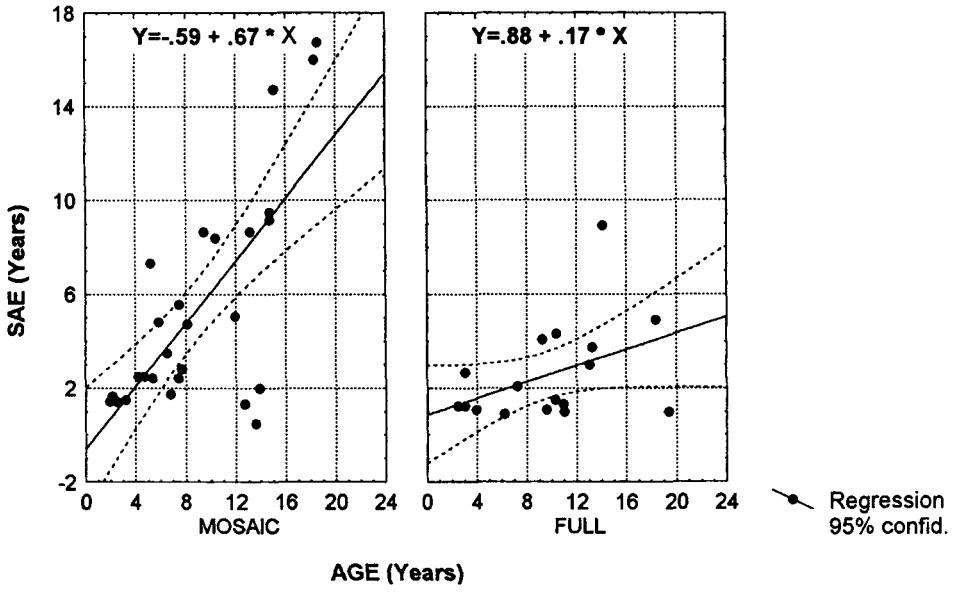

Fig. 3. Socialization age-equivalents (SAE) as a function of age are shown for mosaic and full-mutation males. Solid lines and dashed lines show the best-fitting linear regression and 95% confidence limits for the slopes of the lines, respectively.

Rousseau et al. [1993], IQ was determined by clinical judgment at a large number of institutions, a nonquantitative measure that is not easily interpretable. Variation in technique may have resulted in detection of a lower level of mosaicism in this sample. The number of mosaics identified and the nature of the IQ data may have masked any differences between mosaic and full-mutation cases. In the study by deVries et al. [1993], only standard scores were examined. In addition, age effects were examined in a categorical rather than a continuous manner, with the sample divided into those above and below age 14 years and IQs correlated with the smallest detectable insert size within these two age groups. Our data suggest that adaptive skill development is still growing up to age 20 years, and that these developmental changes could obscure any relationship that exists between IQ and degree of mosaicism. In the study by McConkie-Rosell et al. [1993], all subjects were adults, thus minimizing effects associated with development. This may explain why this was the only published study to demonstrate effects of mosaicism on IQ. However, as noted above, the marked avoidance behaviors noted in this group can make reliable determinations of intellectual functioning difficult to ascertain. It is apparent that future studies that investigate the relationship between degree of mosaicism and intelligence or adaptive functioning should take developmental factors (age, age-equivalents, or mental age) and testing conditions into account in their data analyses.

Implications for prognosis and for the therapeutic role of the *FMR1* gene or protein replacement therapies are complex. The mosaic cases were observed to show, cross-sectionally, 2–4 times the rate of progress of those cases with the full mutation, with the rate of growth over this age span described fairly well by a linear function with similar intercepts across groups. Therefore, since both groups start off at similar levels of adaptive development, at least as detected by the Vineland scales, it is clear that such therapies may have an effect and that the *FMR1* protein may play a role in development during this period. However, it is also possible that the *FMR1* protein has its major effect in embryonic development and that effects in later development are secondary.

In contrast, it is virtually impossible to apply these findings to prenatal diagnosis. In chorionic villus samples (CVS), methylation of *FMR1* is often incomplete. Since the distinguishing feature of a premutation allele is its lack of methylation, CVS DNA is not reliably informative for mosaic status. Similarly, it is not clear that the mosaic status of amniotic fluid-cell DNA is a reliable indicator of postnatal status of neural tissue.

Our data imply that the presence of some *FMR1* protein has a beneficial effect on adaptive skills development among males with this disorder. They also imply that mosaicism detected in leukocytes is usually an accurate reflection of mosaicism in the central nervous system. There are some data suggesting that the proportion of mosaic cells differs in different tissues [Dobkin et al., 1996; Verheij et al., 1993]. Further, selection may favor leukocytes which produce the *FMR1* protein, and thus the level of mosaicism in these cells may not necessarily reflect the degree of mosaicism of neural tissue. Our data suggest that if these factors play a role, they are likely to occur in relatively few individuals, since most older mosaics were functioning at higher levels of development than their full-mutation cohorts. However, variability in behavioral expression within the mosaic and full-mutation groups was observed that could not be attributed to age or mosaic status in leukocytes. Dobkin et al. [1996] have reported, in a small sample, that adaptive behavior is correlated to

a greater extent with mosaicism in skin cells, rather than in blood cells. It is therefore likely that examination of mosaicism in tissues more closely related, embryologically, to brain tissue, may be helpful in understanding some of the variability that we observed in our mosaic and full-mutation groups.

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REFERENCES

American Psychiatric Association (1987): "Diagnostic and Statistical Manual of Mental Disorders," (3rd ed., revised.) Washington, DC: APA, pp 33–39.

Ashley CT, Wilkinson KD, Reines D, Warren ST (1993): FMR1 protein: Conserved RNP family domains and selective RNA binding. Science 262:563–566.

Belser RC, Sudhalter V (1995): Arousal difficulties in males with fragile X syndrome: A preliminary report. Dev Brain Dys 8:270–279.

Brown WT, Jenkins EC (1992): The fragile X syndrome. Mol Genet Med 2:39–66.

Brown WT, Houck GE, Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Sklower Brooks S, Jenkins E (1993): Fragile X carrier screening and prenatal diagnosis using a rapid, non-radioactive PCR test. JAMA 270:1569–1575.

Cohen IL, Fisch GS, Sudhalter V, Wolf-Schein EG, Hanson D, Hagerman R, Jenkins EC, Brown WT (1988): Social gaze, social avoidance and repetitive behavior in fragile X males: A controlled study. Am J Ment Retard 92:436–446.

Cohen IL, Vietze PM, Sudhalter V, Jenkins EC, Brown WT (1989): Parent-child dyadic gaze patterns in fragile X males and in non-fragile X males with autistic disorder. J Child Psychol Psychiatry 30:845–856.

Cohen IL, Vietze P, Sudhalter V, Jenkins EC, Brown WT (1991): Effects of age and communication level on eye contact in fragile X males and nonfragile X autistic males. Am J Med Genet 38:498–502.

De Boulle KD, Verkerk AJMH, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van Den Bos B, de Graaf E, Oostra BA, Willems PJ (1993): A point mutation in the FMR-1 gene associated with fragile X mental retardation. Nat Genet 3:31–35.

DeVries BBA, Wiegers AM, de Graaf E, Verkerk AJMH, Van Hemel JO, Halley DJJ, Fryns J-P, Curfs LMG, Niermeijer MF, Oostra BA (1993): Mental status and fragile X expression in relation to FMR-1 gene mutation. Eur J Hum Genet 1:72–79.

Dobkin CS, Nolin, SL, Cohen IL, Sudhalter VS, Bialer MG, Ding X-H, Jenkins EC, Zhong N, Brown WT (1996): Tissue differences in fragile X mosaics: Mosaicism in blood cells may differ greatly from skin. Am J Med Genet 64:296–301.

Dykens E, Hodapp RM, Ort SI, Leekman JF (1993): Trajectory of adaptive behavior in males with fragile X syndrome. J Autism Dev Disord 23:135–145.

Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJMH, Holden JJA, Fenwick RG, Jr, Warren ST, Oostra AA, Nelson DL, Caskey CT (1991): Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell 67:1047–1058.

Lachiewicz A, Gullian C, Spridigliozzi G, Aylsworth A (1987): Declining IQs of young males with the fragile X syndrome. Am J Ment Retard 92:272–278.

McConkie-Rosell A, Lachiewicz AM, Spiridigliozi GA, Tarleton J, Schoenwald S, Phelan MC, Goonewarenda P, Ding X, Brown WT (1993): Evidence that methylation of the FMR-1 locus is responsible for variable phenotypic expression of the fragile X syndrome. Am J Hum Genet 53:800–809.

Nolin SL, Glicksman A, Houck GE, Brown WT, Dobkin CT (1994): Mosacism in fragile X affected males. Am J Med Genet 51:509–512.

Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991): Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66:817–822.

Rousseau F, Heitz D, Tarleton J, MacPherson J, Malmgren H, Dahl N, Barnicoat A, Mathew C, Mornet É, Tejada I, Maddalena A, Spiegel R, Schinzel A, Marcos JAG, Schorderet DF, Schaap T, Maccioni L, Russo S, Jacobs PA, Schwartz C, Mandel JL (1993): A multicenter study on geneotype-phenotype correlations in the fragile X syndrome, using direct diagnosis with probe StB12.3: The first 2253 cases. Am J Hum Genet 55:225–237.

Silverstein AB (1986): Nonstandard standard scores on the Vineland Adaptive Behavior Scales. A cautionary note. Am J Ment Defic 91:1–4.

Sparrow SS, Balla DA, Cicchetti DV (1984): "Vineland Adaptive Behavior Scales. Interview Edition. Survey Form Manual." Circle Pines, MN: American Guidance.

Verheij C, Bakker CE, de Graaf B, Kuelemans J, Willemsen R, Verkerk AJMH, Galjaard H, Reuser AJJ, Hoogeveen AT, Oostra BA (1993): Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. Nature 363:722–724.